These results indicate that UCP3 influences whole animal metabolism and, at the same time, it can be considered one of the molecular determinant of the metabolic adaptations induced by T3.

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5P8

Uncoupling protein UCP3 up-regulation during cardioplegia-induced ischemia in the human heart

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Uncoupling proteins (UCPs) comprise a subfamily of mitochondrial inner membrane anion carriers that regulate the membrane proton conductance [1]. Although their physiological function is still not established, extensive evidence indicates that they protect against free radical production and oxidative stress [1, 2]. In addition, hypoxia up-regulates UCP3 in skeletal muscle [3] and both UCP2 and UCP3 might confer cardiac ischemia tolerance [4]. Since reactive oxygen species (ROS) also increase during hypoxia [5], the increase in UCP3 could represent a mechanism aimed at decreasing oxidative damage in these conditions. Our aim was to study the effect of cardioplegic arrest-induced ischemia on the expression levels of UCP2 and UCP3 in the human heart. Cardiac biopsies from the left ventricle were obtained from patients undergoing valve replacement surgery with cardiopulmonary bypass. Biopsies were taken before and 20 or 30 min after the infusion of cardioplegic solution. UCP2 and UCP3 mRNA and protein expression levels were determined by quantitative PCR and immunoblot, respectively. UCP3 expression (mRNA and protein) increased during ischemia whereas UCP2 remained unchanged. Ischemia also induced the phosphorylation of ATF-1 (active transcription factor 1) and the nuclear accumulation of the antioxidant transcription factor Nrf2 (NF-E2-related factor 2). Consequently, the mRNA levels of several Nrf2 target genes were also induced. On the contrary, HIF (hypoxia inducible factor) sensitive genes did not change. Relative levels of the five mitochondrial OXPHOS complexes were similar before and after ischemia. Pearson correlation coefficients indicated a positive correlation between UCP3 and Nrf2. The up-regulation of UCP3 during cardiac ischemia could represent a cardioprotective mechanism of mitochondrial depolarization aimed at limiting ROS production.

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5P9

Upregulation of mitochondrial glycerol-3-phosphate dehydrogenase abundance in brown adipose tissue mitochondria from UCP1 knock-out mice

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Uncoupling protein 1 (UCP1) is a mitochondrial inner membrane protein that facilitates heat production in brown adipose tissue and is central to the process of non-shivering thermogenesis in mammals. Furthermore, UCP1 has been demonstrated to have the potential to regulate reactive oxygen containing species (ROS) production by brown adipose tissue mitochondria. One of the crucial sources of electrons for the electron transport chain in brown adipose tissue is via mitochondrial glycerol-3-phosphate dehydrogenase. Mitochondrial glycerol-3-phosphate dehydrogenase is part of the glycerol-phosphate shuttle and links cytoplasmic glycolysis to mitochondrial oxidative phosphorylation. Interestingly, UCP1 knock-out mice showed (a) significantly increased (1.6-fold, $p<0.01$) oxygen consumption rates in brown adipose tissue mitochondria when respiring on glycerol-3-phosphate, (b) increased glycerol-3-phosphate dehydrogenase enzymic activity (1.6-fold, $p<0.001$) and (c) increased abundance of mitochondrial glycerol-3-phosphate dehydrogenase (3-fold, $P=0.003$) per unit mass of mitochondria compared to wild-type controls. Subsequent work will seek to explain the apparent reciprocal arrangement between lack of UCP1 and increased mitochondrial glycerol-3-phosphate dehydrogenase abundance/activity.

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5P10

Molecular characterization of brown adipose tissue in a 'protoendothermic' mammal provides a novel approach to the understanding of uncoupling protein evolution

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The molecular events that facilitated the evolutionary transition from ecto- to endothermia in vertebrates are still unknown. The 'ancient' Lesser hedgehog tenrec, *Echinops telfairi* is considered a 'protoendothermic' mammal as it shows fluctuating, ectothermic-like body temperature patterns. Interestingly, females maintain constantly high body temperatures (~33 °C) during pregnancy and periods of parental care, demonstrating regulatory heat production. Thus, understanding the thermophysiology of this 'protoendotherm' may help to elucidate ancient patterns that led to 'modern'(sustained) endothermy.

We searched for, and characterized the molecular basis of NST in warm (27 °C) and cold (20 °C) acclimated *E. telfairi in vivo* and *in vitro*. Administration of a selective ß3-AR antagonist suppressed rewarming rates from torpor after cold acclimation, indicating involvement of adrenergically mediated nonshivering thermogenesis (NST). Next, morphological analysis revealed a BAT-like depot. The proton leak of isolated BAT mitochondria could be inhibited by GDP, suggesting UCP1-dependent proton conductance and, hence,

the presence of functional BAT, which appeared recruitable by cold acclimation. Molecular analysis confirmed the tissue specific expression of UCP1 in tenrecs (etUCP1). We cloned and stably transfected etUCP1 in HEK293 cells. Isolated mitochondria of etUCP1 HEK293 cells showed inducible proton conductance using palmitate, and GDP-sensitivity, similar to mitochondria containing mouse UCP1. For the search of functional differences, we established bioenergetic measurements in intact HEK293 cells using plate-based respirometry, allowing high-throughput approaches for small molecule modulators. In the initial experiments, we show that a cell-permeable UCP1 activator allows direct specific activation of tenrec and mouse UCP1.

Taken together, we show that *E. telfairi* possesses functional, UCP1-dependent brown adipose tissue, which may facilitate active rewarming from hypothermic states. Substantial evolutionary distance between tenrecs and modern mammals provides a new window to study the evolution of structure-function relationships of UCP1.

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5P11

The breath of a fruit-fly

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The fruit-fly *Drosophila melanogaster* is widely used as a model organism to study human diseases, including those affecting mitochondria. Most mutant flies engineered to mimic mtDNA diseases die at the larval stages, yet a detailed bioenergetic characterization of developmental stage-specific features of Drosophila has never been carried out. We used muscular body-wall Drosophila larvae preparations to study respiration with the sensitive Seahorse[a] technology. This method allows us to study individual larvae and therefore whole tissue bioenergetics *in situ*. Larvae maintained a steady respiratory rate which was inhibited (i) by rotenone and antimycin A, demonstrating its mitochondrial origin and (ii) by 2-iodoacetate, suggesting that respiration is coupled to a high glycolytic flux. Unexpectedly, respiration could not be decreased by the F1FO ATPase inhibitor oligomycin nor increased by the uncoupler FCCP, suggesting that in Drosophila larvae mitochondria are uncoupled. Consistent with a developmental stage-specific uncoupling effect, the respiratory profile of embryonic multilineage Drosophila $S2R^{+}$ cells was instead essentially similar to that of mammalian cells in culture, as basal respiration could be inhibited by oligomycin and then stimulated by FCCP. Sequence homology analysis revealed the existence of four putative uncoupling proteins (UCPs) in Drosophila (UCP4a, UCP4b, UCP4c, and UCP5) that share 60–70% homology with their mammalian counterparts. Whether these are developmentally regulated is not known. We suspect that in the poikilotherm Drosophila uncoupling may be essential for thermogenesis in the pre-pupal stages. We are carrying out silencing of each UCP transcript in whole larvae, as well as genetic ablation of individual UCP genes, to address the potential role of UCP proteins in larval respiration.

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5P12

UCP2 is associated with a high cell proliferative potential and therefore not present in neurons

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Since its discovery 15 years ago the transport function and the involvement of the inner mitochondrial membrane uncoupling protein 2 (UCP2) in diabetes, obesity, arteriosclerosis, neurodegenerative and neuroinflammatory diseases is controversially discussed. Moreover its tissue distribution is still uncertain, especially the presence of the protein in brain. Here, we re-evaluate the UCP2 expression pattern at mRNA and protein level and reveal a strong association of UCP2 with cells and tissues of the immune system, in particular with T-, B-, NK- cells and monocytes. Activation of T-cells leads to a 10-fold increase in UCP2 abundance. The late onset of UCP2 up-regulation in activated T-cells indicates a role of UCP2 in the later events of the immune response like metabolism increase and cell proliferation. We found only UCP4 (1, 2) and not UCP2 in neurons, although we confirmed the presence of UCP2 mRNA in brain. Instead, we detected UCP2 in microglia cells. Together with the reports from other research groups showing the presence of UCP2 in stem and cancer cells, our present results support the idea that UCP2 up-regulation is associated with the proliferative potential of cells.

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5P13

UCP2 in tumour cells: Analyzing its role in the defence against oxidative stress

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The biochemical activity of the uncoupling proteins (UCPs) is to lower the efficiency of the oxidative phosphorylation presumably by increasing the membrane proton conductance. This mild uncoupling would lead to an increase in respiration that should result in a lower generation of superoxide. This uncoupling activity has been proposed to constitute a mechanism of defence against oxidative stress and, in fact, it has been reported that UCPs are upregulated in physiological situations where there is oxidative stress and, furthermore, that their overexpression reduces ROS damage.

Tumour cells have a high intrinsic level of oxidative stress and, in these cells, UCP2 could play a defensive role [1]. Thus, it has been shown that in colon cancer UCP2 expression is increased and that